

WHAT IS CLAIMED IS:

1           1. An isolated infectious recombinant respiratory  
2 syncytial virus (RSV) comprising a RSV genome or antigenome, a  
3 major nucleocapsid (N) protein, a nucleocapsid phosphoprotein  
4 (P), a large polymerase protein (L), and a RNA polymerase  
5 elongation factor, wherein the recombinant RSV has at least two  
6 attenuating mutations, one of the mutations specifying a  
7 temperature-sensitive substitution at amino acid Phe<sub>521</sub>, Gln<sub>831</sub>,  
8 Met<sub>1169</sub>, or Tyr<sub>1321</sub> in the RSV polymerase gene or a temperature-  
9 sensitive nucleotide substitution in the gene-start sequence of  
10 gene M2.

1           2. The RSV of claim 1, having at least three  
2 attenuating mutations.

1           3. The RSV of claim 1, wherein Leu is substituted at  
2 Phe<sub>521</sub> or Gln<sub>831</sub>.

1           4. The RSV of claim 1, wherein Val is substituted at  
2 Met<sub>1169</sub>.

1           5. The RSV of claim 1, wherein Asn is substituted at  
2 Tyr<sub>1321</sub>.

1           6. The RSV of claim 1, wherein at least two of the  
2 mutations are selected from the group consisting of  
3 temperature-sensitive substitutions at Phe<sub>521</sub>, Gln<sub>831</sub>, Met<sub>1169</sub>, and  
4 Tyr<sub>1321</sub>.

1           7. The RSV of claim 6, wherein the  
2 temperature-sensitive substitutions are at Phe<sub>521</sub> and Met<sub>1169</sub>.

1           8. The RSV of 6, wherein the temperature-sensitive  
2 substitutions are at Gln<sub>831</sub> and Tyr<sub>1321</sub>.

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1           9. The RSV of claim 1, wherein two mutations occur in  
2 the codon encoding a temperature-sensitive substitution at  
3 Phe<sub>521</sub>, Gln<sub>831</sub>, Met<sub>1169</sub>, or Tyr<sub>1321</sub>.

1           10. The RSV of claim 1, formulated in a dose of 10<sup>3</sup>  
2 to 10<sup>6</sup> PFU of attenuated virus.

1           11. The RSV of claim 1 further comprising a  
2 nucleotide modification specifying a phenotype selected from a  
3 change in growth characteristics, attenuation,  
4 temperature-sensitivity, cold-adaptation, small plaque size,  
5 host range restriction, or a change in immunogenicity.

1           12. The RSV of claim 11, wherein a SH, NS1, NS2 or G  
2 gene is modified.

1           13. The RSV of claim 12, wherein the SH gene is  
2 deleted.

1           14. The RSV of claim 12, wherein the NS2 gene is  
2 reciprocally substituted in position for the SH gene.

1           15. The RSV of claim 11, wherein said further  
2 nucleotide modification is to a cis-acting regulatory sequence  
3 that is modified or rearranged within the RSV genome or  
4 antigenome.

1           16. The RSV of claim 15, wherein the cis-acting  
2 regulatory sequence is replaced by a heterologous regulatory  
3 sequence.

1           17. The RSV of claim 16, wherein the heterologous  
2 regulatory sequence is a cis-acting regulatory sequence of a  
3 different RSV gene.

1           18. The RSV of claim 11, wherein the nucleotide  
2 modification is selected from a termination codon introduced  
3 within a selected gene or a change in sequence, position, or

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4 presence of a GS or GE transcription signal relative to a  
5 selected gene.

1 19. The RSV of claim 11, wherein the nucleotide  
2 modification comprises an inserted, deleted or altered  
3 translational start codon in sequence context within the RSV  
4 genome or antigenome.

1 20. The RSV of claim 11, wherein a gene or gene  
2 segment encoding an immunogenic F or G protein region of one RSV  
3 subgroup virus is incorporated within a genome or antigenome of  
4 a different RSV subgroup virus.

1 21. The RSV of claim 11, wherein the RSV genome or  
2 antigenome is modified to encode a non-RSV molecule selected  
3 from a cytokine, a T-helper epitope, a restriction site marker,  
4 or a protein of a microbial pathogen capable of eliciting a  
5 protective immune response in a mammalian host.

1 22. The RSV of claim 11, which comprises a gene or  
2 gene from PIV replacing a corresponding gene or gene segment of  
3 RSV.

1 23. The RSV of claim 22, wherein the PIV gene or gene  
2 segment encodes HN or F glycoprotein.

1 24. The RSV of claim 23, wherein the gene segment  
2 encodes a cytoplasmic tail, transmembrane domain or ectodomain  
3 of HN or F of PIV1, PIV2, or PIV3.

1 25. The RSV of claim 11, further comprising a  
2 polynucleotide sequence encoding an immunogenic epitope or  
3 protein region of PIV whereby the RSV elicits an immunogenic  
4 response to both PIV and RSV antigens.

1 26. An isolated infectious RSV particle which  
2 comprises a recombinant RSV genome or antigenome, a major  
3 nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a

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4 large (L) polymerase protein, and a RNA polymerase elongation  
5 factor, wherein the genome or antigenome is modified to ablate  
6 or modulate expression of a SH, NS1, NS2 or G gene or a  
7 cis-acting regulatory sequence.

1 27. The RSV of claim 26, wherein the SH gene is  
2 deleted.

1 28. The RSV of claim 26, wherein the NS2 gene is  
2 reciprocally substituted in position for the SH gene.

1 29. The RSV of claim 26, wherein the cis-acting  
2 regulatory sequence is replaced by a heterologous regulatory  
3 sequence.

1 30. The RSV of claim 29, wherein the heterologous  
2 regulatory sequence is a cis-acting regulatory sequence of a  
3 different RSV gene or of PIV.

1 31. The RSV of claim 26, wherein the RSV genome or  
2 antigenome further comprises a gene or gene segment encoding an  
3 immunogenic F or G protein region of a different RSV subgroup.

1 32. The RSV of claim 26, wherein the genome or  
2 antigenome comprises a chimera of a human RSV sequence and at  
3 least one non-human RSV sequence.

1 33. The RSV of claim 26, wherein the genome or  
2 antigenome encodes a human RSV in which a selected gene or gene  
3 segment is replaced with a counterpart gene or gene segment from  
4 a heterologous RSV.

1 34. The RSV of claim 33, wherein the selected gene is  
2 NS1 or NS2 and the counterpart gene is N.

1 35. The RSV of claim 26, wherein the RSV genome or  
2 antigenome further comprises a nucleotide sequence of a non-RSV  
3 molecule selected from a cytokine, a T-helper epitope, a

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restriction site marker, or a protein of a microbial pathogen capable of eliciting a protective immune response in a mammalian host.

36. The RSV of claim 26, which further comprises a gene or gene from PIV replacing a corresponding gene or gene segment of RSV.

37. The RSV of claim 36, wherein the PIV gene or gene segment encodes HN or F glycoprotein of PIV1, PIV2, or PIV3.

38. An isolated infectious RSV particle which comprises a recombinant RSV genome or antigenome, a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large (L) polymerase protein, and a RNA polymerase elongation factor, wherein the RSV genome or antigenome is modified by a termination codon introduced within a selected gene, or by a change in sequence, position, or presence of a GS or GE transcription signal relative to the selected gene.

39. A method for stimulating the immune system of an individual to induce protection against respiratory syncytial virus, which comprises administering to the individual an immunologically sufficient amount of the isolated attenuated recombinant RSV of claim 1, 26 or 38 in a physiologically acceptable carrier.

40. The method of claim 39, administered in a dose of  $10^3$  to  $10^6$  PFU of the attenuated RSV.

41. The method of claim 39, wherein the attenuated RSV is administered to the upper respiratory tract.

42. The method of claim 39, wherein the attenuated RSV is administered by spray, droplet or aerosol.

43. The method of claim 39, wherein the attenuated RSV is administered to an individual seronegative for antibodies

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3 to RSV or possessing transplacentally acquired maternal  
4 antibodies to RSV.

1 44. A vaccine to induce protection against RSV, which  
2 comprises an immunologically sufficient amount of the isolated  
3 attenuated recombinant RSV of claim 1, 26 or 38 in a  
4 physiologically acceptable carrier.

1 45. The vaccine of claim 44, formulated in a dose of  
2  $10^3$  to  $10^6$  PFU of the attenuated RSV.

1 46. The vaccine of claim 44, formulated for  
2 administration to the upper respiratory tract by spray, droplet  
3 or aerosol.

1 47. A composition which comprises an expression  
2 vector comprising an isolated polynucleotide molecule encoding a  
3 RSV genome or antigenome having at least two attenuating  
4 mutations, at least one of the mutations specifying a  
5 temperature-sensitive substitution at amino acid Phe<sub>521</sub>, Gln<sub>831</sub>,  
6 Met<sub>1169</sub>, or Tyr<sub>1321</sub> in the RSV polymerase gene or a temperature-  
7 sensitive nucleotide substitution in the gene-start sequence of  
8 gene M2, and one or more expression vector which comprises one  
9 or more polynucleotide molecules encoding N, P, L and RNA  
10 polymerase elongation factor proteins of RSV, whereby upon  
11 expression an infectious RSV particle is produced.

1 48. The composition of claim 47, wherein the  
2 infectious RSV particle is a virus.

1 49. A method for producing an infectious attenuated  
2 RSV particle from one or more isolated polynucleotide molecules  
3 encoding said RSV, comprising:

4 coexpressing in a cell or cell-free lysate the  
5 expression vectors of claim 47, thereby producing said  
6 infectious RSV particle.

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1 50. The method of claim 49, wherein the RSV genome or  
2 antigenome and the N, P, L and RNA polymerase elongation factor  
3 proteins are expressed by two or more different expression  
4 vectors.

1 51. The method of claim 49, wherein the RSV wherein  
2 the genome or antigenome is modified to ablate or modulate  
3 expression of a SH, NS1, NS2 or G gene or a cis-acting  
4 regulatory sequence.

1 52. The method of claim 51, wherein the SH gene is  
2 deleted.

1 53. The method of claim 51, wherein the NS2 gene is  
2 reciprocally substituted in position for the SH gene.

1 54. The method of claim 51, wherein the cis-acting  
2 regulatory sequence is replaced by a heterologous regulatory  
3 sequence.

1 55. The method of claim 54, wherein the heterologous  
2 regulatory sequence is a cis-acting regulatory sequence of a  
3 different RSV gene or of PIV.

1 56. The method of claim 51, wherein the RSV genome or  
2 antigenome further comprises a gene or gene segment encoding an  
3 immunogenic F or G protein region of a different RSV subgroup.

1 57. The method of claim 51, wherein the genome or  
2 antigenome comprises a chimera of a human RSV sequence and at  
3 least one non-human RSV sequence.

1 58. The method of claim 51, wherein the genome or  
2 antigenome encodes a human RSV in which a selected gene or gene  
3 segment is replaced with a counterpart gene or gene segment from  
4 a heterologous RSV.

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1 59. The method of claim 51, wherein the RSV genome or  
2 antigenome further comprises a nucleotide sequence of a non-RSV  
3 molecule selected from a cytokine, a T-helper epitope, a  
4 restriction site marker, or a protein of a microbial pathogen  
5 capable of eliciting a protective immune response in a mammalian  
6 host.

1 60. The method of claim 51, wherein the RSV genome or  
2 antigenome further comprises a PIV gene or gene segment encoding  
3 HN or F glycoprotein of PIV1, PIV2, or PIV3.

1 61. An RSV strain selected from *cpts* RSV 248 (ATCC VR  
2 2450), *cpts* 248/404 (ATCC VR 2454), *cpts* 248/955 (ATCC VR 2453),  
3 *cpts* RSV 530 (ATCC VR 2452), *cpts* 530/1009 (ATCC VR 2451), or  
4 *cpts* 530/1030 (ATCC VR 2455).

1 62. An RSV strain selected from B-1 *cp*52/2B5 (ATCC VR  
2 2542) or B-1 *cp*-23 (ATCC VR).

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